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Chapter 13

CELL INTERACTION WITH CELLULOSE-BASED SCAFFOLDS FOR TISSUE ENGINEERING: A REVIEW

Lucie Bacakova[1,*], Katarina Novotna[1], Tomas Sopuch[2] and Pavel Havelka[3]

[1]Department of Biomaterials and Tissue Engineering,
Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic
[2]Holzbecher s.r.o., Ceska Skalice - Zlic, Czech Republic
[3]Lonza Biotec s.r.o., Kourim, Czech Republic

ABSTRACT

Cellulose is a structural polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units. This natural polymer is synthesized by herbs, wooden plants, many forms of algae, fungi and some species of bacteria. Cellulose has been widely used in biomedical applications, including clinical applications as wound dressings, carriers for drug delivery, preparations for treatment of ophthalmological disorders, membranes for prevention of postoperative adhesions, meshes for hernia repair, materials for hemostasis, membranes for hemodialysis, and also as materials for plastic, reconstructive and aesthetic surgery. In addition, cellulose-based materials have been experimentally tested as cell carriers for tissue engineering, and some of these results have been introduced into clinical practice. This review summarizes the status of the use of cellulose-based materials over the past 30 years for engineering, reconstruction and regeneration of various tissues, namely blood vessels, cardiac muscle, heart valves, skeletal muscle, skin, liver, pancreatic islets, the peripheral nervous system, the central nervous system, the urinary system, bone, cartilage, tendon and ligament. The experience of our group in vascular and bone tissue engineering using cellulose-based materials, such as viscose, dialdehyde cellulose, cotton and 6-carboxycellulose, is also included.

Keywords: polysaccharides, cellulose, biomaterial, tissue engineering, regenerative medicine, cell therapy

E-mail: Lucie.Bacakova@fgu.cas.cz.

INTRODUCTION

Cellulose is a structural polysaccharide consisting of a linear chain of several hundred to over ten thousand β(1→4) linked D-glucose units. It was discovered and isolated from green plants by Payen [1] (for a review, see [2]). It is the most abundant biopolymer on Earth, synthesized by herbs, wooden plants, many forms of algae, fungi and some species of bacteria, namely by *Gluconacetobacter xylinus* (formerly referred to as *Acetobacter xylinum*). Bacterial cellulose is identical to plant cellulose in chemical structure, but it can be produced without contaminant molecules, such as lignin and hemicelluloses, and does not require intensive purification processes. In addition, it is remarkable for its mechanical strength and biocompatibility, so it is suitable for biomedical applications, particularly for tissue engineering, where the cell carriers should not only to be well-tolerated by the organism, but they should also match the mechanical properties of the replaced tissue (for a review, see [3]).

Cellulose-based materials have achieved a remarkably wide range of applications in clinical practice. These materials serve as wound dressings, carriers for drug delivery, preparations for treatment of ophthalmological disorders, membranes for prevention of postoperative adhesions, meshes for hernia repair, materials for hemostasis, membranes for hemodialysis, and also as materials for plastic, reconstructive and aesthetic surgery.

Cellulose-based wound dressings have been applied for treating acute and chronic skin wounds, such as burns [4], leg venous ulcers [5], and immune (allergic) disorders [6, 7]. For treatment of burns, a viscose cellulose sponge Cellonex™ was used. Although this material evoked some inflammatory reaction, it induced earlier vascularization than Integra®, a commonly used material in skin wound treatment [4]. In the case of leg venous ulcers, there was a more improved periulcer skin condition using a biosynthetic cellulose dressing than when standard care with a foam dressing was applied [5]. An advanced wound dressing made of crystalline cellulose (Veloderm) accelerated healing of skin wounds caused by burns or by reconstructive plastic surgery, and also required a dressing change less frequently than Vaseline gauze, which had traditionally been applied [8]. Oxidized regenerated cellulose accelerated re-epithelialization of oral mucosal lesions in comparison with conventional medication [9]. Cellulose materials have been combined with antimicrobial agents, such as antibiotics [10] or silver [11], in order to prevent secondary infection of wounds, and thus to accelerate healing. Clothing made of cellulose fibers enriched with silver ions [6], and also citric acid-coated cellulose textiles improved healing and the barrier function of skin affected by atopic eczema [7].

Well-known cellulosic materials used in the moist wound care are hydrocolloids (usually a mixture of sodium salt of carboxymethylcellulose with pectin and gelatin) [12], hydrocolloidal fibers from sodium salt of carboxymethylcellulose [13] known as Aquacel® (Convatec), or there is also a similar product Durafiber® (Smith and Nephew). Durafiber® is a non-woven fabric made of the mixture of cellulose and cellulose ethylsulfonate [14]. Both Durafiber® and Aquacel®, creating soft gel on the wound, are also available in the form of silver-containing antimicrobial dressing. The acidic carboxymethylated cellulose wound dressing available as Hcel® HT (Holzbecher) [15].

In addition to their skin care applications, cellulose-based materials have also been used as carriers for delivering drugs into other tissues, including tumors. For example, hydroxypropyl methylcellulose capsules containing Dabrafenib were administered orally to

patients to treat BRAF V600 mutation-positive melanoma, and these capsules were shown to dissolve a higher percentage of dabrafenib than gelatin capsules [16]. Vaccination with glioma-associated antigen peptides stabilized in carboxymethylcellulose was used for treatment of gliomas in child patients [17]. Orally disintegrated films containing hydroxypropyl methylcellulose facilitated oral administration of drugs (donepezil) in Alzheimer disease patients [18]. Hydroxypropyl methyl cellulose, Carbopol 934, served for delivering an antimicrobial agent metronidazole for treatment of periodontal diseases [19].

Ophthalmology is another important field for the clinical application of cellulose-based materials. Hydroxypropyl cellulose ophthalmic inserts (LACRISERT®) have been used successfully for treating the dry eye syndrome occurring e.g., in eye lens wearers and during autoimmune diseases [20, 21]. In addition, lubricant eye drops (Can-C) designed as 1% N-acetylcarnosine prodrug of L-carnosine containing a mucoadhesive cellulose-based compound combined with corneal absorption promoters improved the vision in patients with age-related cataracts, and thus they have potential for non-surgical treatment of this disorder [22].

Cellulose-based materials efficiently prevented postoperative adhesions after gynaecological, abdominal and lumbar surgery. Oxidized regenerated cellulose (Interceed) adhesion reduced the incidence of pelvic adhesion formation in both laparoscopy and laparotomy. Chemically modified sodium hyaluronate/carboxymethylcellulose (Seprafilm) was effective in preventing adhesion formation, especially following myomectomies [23]. Bioresorbable hyaluronic acid/carboxymethylcellulose membrane prevented abdominal and perihepatic adhesions in metastatic colorectal cancer patients requiring 2-stage hepatectomy [24]. Hyaluronate-carboxymethylcellulose also reduced the incidence of reoperations for adhesive small bowel obstruction [25], and showed beneficial anti-adhesive and anti-inflammatory effects after tympanomastoid surgery, resulting in postoperative hearing improvement [26]. Carboxymethylcellulose/polyethylene oxide gel reduced posterior dural adhesions in the spine, lower-back pain and leg pain in patients with lumbar discectomy for herniated lumbar discs [27]. Sheets of regenerated oxidized cellulose (Tabotamp) with bupivacaine significantly reduced pain after video-laparoscopic cholecystectomy and significantly reduced the use of postoperative pain killers [28]. A composite mesh with incorporated oxidized regenerated cellulose (Proceed) was used for minimally invasive laparoscopic repair of ventral hernia [29].

For hemostasis, cellulose-based materials have been used in the form of a powder or various tissue-like substrates, such a patches, cuffs, bolsters and tampons. A powder made of cellulose proved to be a good haemostatic agent following sinus surgery [30]. The Veriset™ haemostatic patch,i.e., a topical haemostat made of oxidized cellulose and self-adhesive hydrogel components, proved to be effective for hemostasis in patients undergoing hepatic resection. This material achieved hemostasis significantly faster than another clinically applied material, i.e a TachoSil® fibrin sealant patch [31]. Oxidized regenerated cellulose has also been applied in laparoscopy to achieve hemostasis during surgery [32]. Tampons prepared from oxidized regenerated cellulose (EpiCell) were clinically tested in patients with inherited bleeding disorders, such as Glanzmann disease or von-Willebrand disease [33]. Cellulose bolsters were used for hemostasis during laparoscopic partial nephrectomy in patients with renal tumors [34]. Cellulose porous beads were efficient for the preoperative embolization of the vascular bed of meningiomas [35].

Cellulose has been widely used for constructing membranes for hemodialysis, which have been applied in clinical practice. However, these membranes have often been considered to be less biocompatible than membranes made of synthetic polymers, e.g., polysulfone [36-38]. For example, the development of amyloid disease and plasma triglyceride values were higher in patients treated with cellulose membranes than in patients treated with synthetic membranes [36]. However, the biocompatibility of cellulose membranes can be enhanced by various cellulose modifications, e.g., by coating with vitamin E, which reduced circulating biomarkers of lipid peroxidation [39], or by chemical substitution of hydroxyl group of cellulose for the carboxyl group, which converts the cellulose into cellulose triacetate. These membranes have a homogeneous structure and can be produced with a wide range of permeability (i.e., from low-flux performance to super high-flux performance), with high diffusive efficiency and with a uniform pore size distribution [40].

In plastic and reconstructive surgery, oxidized regenerated cellulose has been applied for breast conserving surgery [41], particularly for preventing cosmetic defects in patients undergoing oncoplastic procedures for breast cancer (Tabotamp fibrillar, Johnson, Johnson; Ethicon, USA). In addition to improved cosmetic results, these implants reduced the rate of post-operative bleeding and infection at the surgical site [42]. Carboxymethylcellulose hydrogel implants have several advantages over conventionally used silicone gel, such as higher radiotranslucency and easier insertion through a small incision, because of the highly elastic shell [43]. Carboxymethylcellulose also proved to be an excellent material for correcting facial defects, such as nasolabial folds, perioral wrinkles, and low lip volume, through intradermal injections [44].

Similarly, cellulose has been used for engineering practically all tissues and organs in the mammalian organism. These applications include soft and hard tissue engineering,i.e., blood vessels, cardiac muscle, heart valves, skeletal muscle, skin, liver, pancreatic islets, the peripheral nervous system and the central nervous system, the urinary system (kidneys and bladder), bone, cartilage, tendon and ligament. Although most of these applications were carried out in experimental *in vitro* and *in vivo* systems, some of them have been introduced into clinical practice, e.g., the use of cellulose-based materials for cell therapy of pancreatic cancer in human patients [45], for replacing the *dura mater* covering the brain [46, 47], and for filling bone defects in patients after tooth extraction [48], as will be mentioned in more detail below. Our contribution to tissue engineering of blood vessels and bone on the basis of cellulose will also be mentioned in greater detail [49, 50].

USE OF CELLULOSE IN SPECIFIC FIELDS OF TISSUE ENGINEERING

Cellulose in Vascular Tissue Engineering

One of the first attempts at vascular tissue engineering was carried out with microfibers made of regenerated cellulose, which is purified plant cellulose chemically converted from short fibers into long fibers for use in textiles and nonwovens. These fibers were modified with fibronectin in order to improve cell adhesion, and were successfully applied for constructing three-dimensional vascularized tissue *in vitro* [51]. In vascular tissue engineering, the most frequently used type of cellulose is bacterial cellulose. The research

group of Backdahl et al. investigated three-dimensional nanofibrous bacterial cellulose, which allowed adhesion and proliferation of human saphenous vein smooth muscle cells (SMC) on the surface of and inside the scaffolds [52, 53].

The cellulose-based scaffolds were attractive not only for vascular SMC, but also for vascular endothelial cells. The adhesion, spreading, formation of an actin cytoskeleton and maturation of human saphenous vein cells was supported by nanofibrous bacterial cellulose scaffolds, particularly those functionalized with xyloglucan-bearing RGD-containing oligopeptides,i.e., ligands for integrin adhesion receptors on cells [54, 55]. Similarly, the proliferation of endothelial cells and the spontaneous formation of capillary tube-like structures *in vitro* were improved on nano- and micro-fibrous cellulose acetate scaffolds after they were combined with chitosan [56]. The angiogenic response to cellulose was also observed under *in vivo* conditions,i.e., after implantation of bacterial cellulose scaffolds in the form of a dorsal skinfold chamber into Syrian golden hamsters [57].

Bacterial cellulose has also been used for creating tubular scaffolds to replace small-caliber vessels. The construction of functional small-caliber vascular replacements is relatively complicated, because these grafts are associated with the highest risk of restenosis and failure. Optimal prevention of restenosis is achieved by covering the inner surface of these grafts with endothelial cells. *In vivo* experiments showed that replacement of the carotid arteries with small-diameter bacterial cellulose grafts resulted in the development of a confluent inner endothelial cell layer [58] as well as a layer consisting of SMC [59, 60]. The mechanical properties of tubular structures formed from bacterial cellulose seemed to be advantageous for vascular tissue engineering [61]. In addition, blood compatibility results obtained on vascular grafts made of bacterial cellulose were favourable. These results showed that bacterial cellulose demonstrates no significant difference in platelet consumption, as compared to clinically used poly(ethylene terephthalate) (PET) or expanded polytetrafluoroethylene (ePTFE) [62].

Some novel cellulose nanocomposites have been developed for potential use in vascular tissue engineering. For example, nanocrystalline cellulose and fibrin nanocomposites have been synthesized. The degree of oxidation of nanocrystalline cellulose and the nanocrystalline cellulose-to-fibrin ratio resulted in variable strength and elongation of the nanocomposites [63]. Another example is a scaffold consisting of cellulose nanowhiskers embedded in a matrix of cellulose acetate. This biomaterial delivered excellent mechanical stability [64].

Although these findings are encouraging, cellulose is not an ideal scaffold for tissue engineering in terms of its degradation ability. As mentioned above, ideal scaffolds should be constructed from resorbable materials that degrade in proportion to the regeneration of the tissue [65]. However, cellulose in the organism behaves as a non-degradable or very slowly degradable material. For example, the degradation time of viscose cellulose sponges implanted subcutaneously into rats was longer than 60 weeks [66]. Oxidation is an efficient method for inducing degradability of cellulose [67, 68]; for a review see [50]. Oxidized cellulose is degradable by hydrolysis, mediated by hydrolytic enzymes present in the serum supplement of cell culture media *in vitro* and in macrophages *in vivo* [69, 70]. Cellulose oxidation induces conversion of the glucose residues to glucuronic acid residues containing -COOH groups. The concentration of these groups modulates not just the degradation time of cellulose, but also its pH, its swelling in a water environment, its mechanical stability, drug loading efficiency and other behavior of the material [71]. In addition, the -COOH groups,

which are polar and negatively charged, can be used for functionalizing the oxidized cellulose with various biomolecules [72, 73].

In our experiments, we have focused on preparing and testing cellulose-based materials modified with oxidation and/or functionalization with biomolecules. Namely, 6-carboxycellulose with 2.1 or 6.6 wt.% of –COOH groups was further functionalized with arginine or with chitosan in order to balance the relatively acid character of the oxidized cellulose molecules. Materials were seeded with vascular SMC, and the adhesion, proliferation and phenotypic maturation of the cells was evaluated. We found that oxidized cellulose with 2.1 wt.% of –COOH groups functionalized with chitosan was the most appropriate of all the tested materials for colonization with vascular SMC. This conclusion was based on the highest numbers of cells found on these samples after 7 days of cultivation, either on the material itself, or on the bottoms of polystyrene culture dishes in the presence of this material. Accordingly, the adhered cells were elongated in shape on cellulose with 2.1 wt.% of –COOH groups, while they tended to be spherical in shape on the other materials. In addition, the concentration of contractile proteins alpha-actin and SM1 and SM2 myosins were significantly higher on oxidized cellulose with 2.1 wt.% of –COOH groups. Functionalization of the material with arginine and chitosan further slightly increased the concentrations of these proteins in cells grown on these samples. However, it should be mentioned that the overall proliferation of the vascular SMC on the cellulose materials was low, when compared to the control polystyrene culture dish. However, uncontrolled and massive proliferation of vascular SMC is not desired in vascular tissue engineering [50]. In our earlier study, besides carboxycellulose with 2.1 or 6.6 wt.% of –COOH groups, we also tested viscose and dialdehyde cellulose. The stability of dialdehyde cellulose proved to be very low due to its loose network, which resulted in poor attachment of the cells on this material. Carboxycellulose with 6.6 wt.% of –COOH groups, due to its relatively high acidity was also very unstable in the cell culture system. Viscose, on the other hand, was the most stable of all the tested materials, with almost no tendency to degrade [49]; for a review, see [3].

Another experiment was performed on polyethylene (PE) foils doped with various concentrations (0-20 wt.%) of calcium salt of oxidized cellulose (OKCEL Ca-L, Synthesia, Pardubice, Czech Republic). On all samples doped with oxidized cellulose, the vascular SMC cells proliferated better than on non-doped PE, but the highest cell numbers were found on samples with lower concentrations of oxidized cellulose,i.e., 1-5 wt.% [74].

Cellulose in Cardiac Muscle Regeneration and Engineering

Cellulose based-polymers have been used particularly for stimulating the regeneration and function of myocardial tissue after infarction, which is the main cause of heart failure. For this purpose, cellulose-based materials have been used in the form of injectable hydrogels, which are cell-free [75], containing cells, particularly stem cells [76-78] or containing the extracellular matrix (ECM) from bone marrow, which is considered to stimulate angiogenesis and tissue repair following ischemia-reperfusion injury [79].

Injection of thermo-reversible methylcellulose modified with ECM-derived RGD and HepIII peptides, which bind cell adhesion receptors of the integrin superfamily, into the aneurysmal infarct region of the left ventricle of rats improved left ventricular function,

increased angiogenesis, decreased infarct size, and increased the cardiomyocyte number within the infarct region [75].

A thermo-responsive methylcellulose hydrogel system was also used for fabricating cell sheet fragments containing human amniotic fluid stem cells (hAFSC) with their own ECM. These cell sheet fragments were then injected into the peri-ischemic area of an immune-suppressed rat model after experimentally-induced myocardial infarction. The hAFSC cell sheet fragments had better ability to retain cells, to support cell proliferation, tissue vascularization and to reduce the infarct size than the control dissociated hAFSCs transplanted to the myocardium. In addition, histological and qPCR analyses suggested that the hAFSCs transplanted in the form of cell sheet fragments can be differentiated into cardiomyocyte-like cells and cells of endothelial lineages, and modulated expression of multiple angiogenic cytokines and cardiac protective factor, which improved the ventricular function [76].

Similar results were also obtained when the thermo-responsive methylcellulose hydrogel system was used for constructing spherically symmetric cell bodies containing hAFSCs for cellular cardiomyoplasty [77], or for constructing cell sheet fragments, containing autologous bone marrow-derived mesenchymal stem cells (MSCs) in a porcine model [78]. MSC sheet fragments in infarcted hearts attenuated the adverse ventricular dilation, preserved the cardiac function, prevented scar expansion and left ventricle remodeling. Immunohistochemical analysis demonstrated that the engrafted MSCs can differentiate into endothelial cells and smooth muscle cells, implying that angiogenesis and subsequent regional perfusion improvement is a promising mechanism for ameliorating post-infarcted cardiac function. However, the transplanted MSCs may provoke arrhythmia [78].

Interestingly, ECM alone,i.e., incorporated in cellulose-based gels without cells, proved to be sufficient to induce significant repair of the infarcted myocardial tissue. Injecting bone marrow ECM in a methylcellulose carrier gel in a rat model of myocardial infarction reduced the infarct area, decreased cell apoptosis, improved fractional shortening, enhanced angiogenesis, and led to significantly lower macrophage counts in the infarct border [79].

In experimental cardiac tissue engineering, cellulose has also been used for constructing three-dimensional scaffolds. Cellulose acetate and regenerated cellulose scaffolds were prepared in the form of wavy microscale fibers or three-dimensional grooved topographies by casting the materials onto micromachined surfaces. These scaffolds provided good support for the adhesion and growth of cardiac cells isolated from neonatal rats; the support was comparable with the control polystyrene dishes. In addition, the molding capabilities of the materials down to the nanoscale were comparable with the current favorite in soft lithography,i.e., polydimethylsiloxane, and the scaffold biodegradability can be controlled by hydrolysis, de-acetylization of cellulose acetate and cytocompatible enzyme (i.e., cellulase) action [80].

Cellulose in Heart Valve Reconstruction

Cellulose-based materials have been used relatively rarely for reconstructing heart valves, although these materials are biocompatible and can be elaborated with good mechanical properties. The main problem with polymeric heart valves is that they usually fail in long-term use owing to tearing and calcification of the leaflets under high dynamic tensile bending

stress and due to oxidative reactions with blood. Cellulose-based materials offer the possibility to create artificial valve leaflets which mimic the structure and the mechanical properties of the native valve leaflet. A composite containing polyvinyl alcohol (PVA) matrix reinforced with bacterial cellulose (BC) fibers is used for this purpose. A combination of 15 wt.% PVA and 0.5 wt.% BC seemed to be optimal, because the mechanical properties of this material, evaluated by tensile testing and stress relaxation testing, were similar to those of the porcine heart valve in both the circumferential direction and the axial tissue direction [81-83].

Cellulose in Skeletal Muscle Tissue Engineering

Skeletal muscle is an oriented tissue, so its proper engineering requires oriented substrates. On glass coverslips coated with radially-oriented cellulose nanowhiskers, C2C12 cells, derived from the thigh muscle of C3H mice after a crush injury, adopted oriented morphologies and fused into myotubes, which increased with increasing orientation of the nanowhiskers and was less apparent on the control flat surfaces [84, 85]. Multilayer coatings of polycationic chitosan paired with polyanionic semi-synthetic cellulose sulfates or heparin supported the adhesion and growth of C2C12 cells, which was more apparent on the material with an intermediate degree of sulfation than on highly sulfated materials [86].

Cellulose in Skin Tissue Engineering

Skin treatment products can be divided into two main groups: wound dressings and scaffolds for skin tissue engineering. While wound dressings should be easily removable without causing skin tissue damage, tissue engineering scaffolds should be able to adhere to the wound and support cell proliferation during skin regeneration. These distinct adherence features can be adjusted in composite cellulose acetate/gelatin scaffolds by changing the ratio of cellulose acetate and gelatin. High proliferation of human dermal fibroblasts on electrospun cellulose acetate/gelatin 25:75 confirmed the capability of cellulose acetate/gelatin 25:75 nanofibers as a tissue-engineered scaffold, while the electrospun cellulose acetate/gelatin 75:25 can be a potential low-adherent wound dressing [87].

Other cellulose-based materials supporting the adhesion and growth of dermal fibroblasts are transfer membranes made of enzyme-digestible cellulose [88], 2,3 dialdehyde cellulose hydrogel membranes [89] or composite electrospun nanofibrous scaffolds containing polyurethane, cellulose acetate and zein [90]. Surprisingly, bacterial cellulose supported colonization with fibroblasts to a lower extent than the adhesion, spreading and growth of keratinocytes [91], which was further enhanced by combining cellulose with chitosan [92]. However, in another study performed on degradable transfer membranes, keratinocyte proliferation was lower on membranes based on cellulose and chitosan than on collagen membranes [88].

An ordered cellulose film scaffold, termed a nematic ordered cellulose template, is an interesting material. This material allowed 3D proliferation of human epidermal keratinocyte layers in the perpendicular direction, which was in accordance with the basic concept of skin formation [93]. Other advanced growth supports for keratinocytes are porous three-

dimensional hydrogel matrices composed of carboxymethylcellulose and a silk cocoon protein sericin [94], or nanofibrous composites of cellulose, silk fibroin and lysozyme [95].

Cellulose and Liver Engineering

In the first phase of the application of cellulose-based materials in liver replacement, these materials were used for encapsulating hepatocytes for constructing extracorporeal bioartificial liver support systems in order to improve the condition of patients with hepatic failure, and to survive the period until a suitable donor liver is available. In addition, this technology enables the use of hepatocytes of allogenic or xenogenic origin, or even in the form of tumor-derived cell lines. For this purpose, a material composed of polysaccharides (carboxymethylcellulose, chondroitin sulfate A, chitosan, and polygalacturonate) was developed, and was found to be superior to widely used alginate-polylysine capsules [96, 97]. Murine hepatocytes encapsulated in permeable multicomponent capsules, formed by polyelectrolyte complexation between sodium alginate, cellulose sulfate and poly(methylene-co-guanidine) hydrochloride, retained their specific functions, namely transaminase activity, urea synthesis, and protein secretion during the first four days of culture in a minimum medium. The technology for encapsulating hepatocytes is also useful for fundamental research, e.g., in analyses of drug metabolism, intercellular regulations, and metabolic pathways, and also for establishing a tissue bank for storing and supplying hepatocytes [98]. Biodegradable microcapsules containing cellulose were used for engineering vascularized liver tissue *in vitro*. In these experiments, hepatocytes were suspended in glycosaminoglycan (GAG) solutions (4%/1.5% chondroitin sulfate/carboxymethylcellulose, or 1.5 wt.% hyaluronan) and encapsulated by forming chitosan-GAG polyelectrolyte complex membranes around droplets of the cell suspension [99].

Cellulose-based microcarriers in the form of porous micro-sized membranes [100] and microspheres [101] have also been applied for constructing a bioartificial liver support. Multiporous membrane-like cellulose microcarriers were used for immobilizing hepatocytes and for studies on their metabolic activity in a floating culture, in a newly developed bioreactor, and under perfusion of a hollow-fiber-based hybrid artificial liver support system [100]. Cellulose microspheres containing cell-adhesive Gly-Arg-Gly-Asp-Ser (GRGDS) peptides,i.e., ligands for cell adhesion receptors, excellently immobilized hepatocytes on the surface of microspheres in a high number and quality [101]. Cellulose beads were used for reconstructing liver organoids in a radial-flow bioreactor using a functional human hepatocellular carcinoma cell line FLC-5 as hepatocytes, mouse immortalized sinusoidal endothelial cell line M1 and mouse immortalized hepatic stellate cell (line A7) as non-parenchymal cells [102]. Cellulose acetate modified with 2-methacryloyloxyethyl phosphorylcholine copolymers was used for constructing a hollow fiber membrane bioreactor for use in an extracorporeal therapy. Modification with these copolymers increased the functionality of hepatocytes in terms of urea synthesis and albumin synthesis [103].

Another three-dimensional system which promoted phenotypic maturation and function of hepatocytes consisted of wood-derived nanofibrillar cellulose combined with hyaluronan-gelatin hydrogels. This material induced the formation of 3D multicellular spheroids of HepaRG liver progenitor cells, characterized by apicobasal polarity and functional bile canaliculi-like structures, i.e., structural hallmarks of liver tissue. In addition, the cells

expressed the mRNA for hepatocyte markers albumin and cytochrome P450 3A4 (CYP3A4), and showed metabolic activity of this enzyme [104, 105]. A thin 3D-microstructured fibrous substrate consisting of a microfibrillated cellulose sheet coating a highly O_2-permeable polydimethylsiloxane (PDMS) membrane proved to be suitable for obtaining stably-attached and functional rat hepatocyte 3D cultures in the form of hemispheroids, and appeared interesting for drug/chemical screenings in a microplate format, and also for microfluidic applications [106].

Planar cellulose membranes and sheets also provided good supports for cultures of hepatocytes. Some of the first materials used for this purpose were Cuprophan cellulose membranes. When coated with collagen or fibronectin, these membranes induced spherical or polygonal flattening of hepatocytes, as confirmed by computer-aided time-lapse video analysis [107]. Flat sheet membranes made of cellulose acetate or aminated cellulose acetate supported the adhesion, viability and function of rat hepatocytes (manifested by urea synthesis and ammonia utilization), though to a lower extent than polysulfone-based membranes [108]. The adhesion and growth of hepatocytes on cellulose acetate membranes was also positively influenced by the free surface energy, wettability and surface tension of these membranes. This is a typical feature of the cell behavior in cultures on various biomaterials [109]. Other cellulose-based substrates suitable for hepatocyte cultivation were carboxymethylcellulose membranes, particularly due to their mechanical and cell-interaction properties [110], and lactose-modified cellulose films, which promoted hepatocyte adhesion through a direct interaction between galactose residues on the cellulose films and asialoglycoprotein receptors on the cell surface [111].

Cellulose and Pancreatic Islet Delivery

Cellulose-based or cellulose-containing materials have been used massively for encapsulation, immunoisolation, cryoprotection and long-term storage (banking) of Langerhans islets for transplantation. The first experiments in this field started as long as 30 years ago (in 1985), when cellulose sulfate and regenerated cellulose were used for cultivating pancreatic islets, and for encapsulating them and implanting them into rats.

Pancreatic islets cultured in the presence of 2% cellulose sulfate for up to 3 weeks are characterized by unchanged insulin content, secretion and biosynthesis when compared to appropriate controls [112]. The application of cellulose sulfate in the combination of sodium alginate, poly(methylene-co-guanidine) hydrochloride, calcium chloride, and sodium chloride proved to be particularly promising for pancreatic islet encapsulation from the point of view of islet viability, immunoisolation, insulin secretion and the mechanical strength of the capsules, which was markedly superior to the widely-used alginate/poly(L-lysine) capsules [113, 114]. Recently, sodium cellulose sulfate has been tested for islet cryopreservation and banking [115], and particularly for cell therapy of pancreatic cancer in human patients. In a clinical trial performed on patients with non-resectable pancreatic cancer, genetically modified 293-derived cells overexpressing a cytochrome P450 enzyme were encapsulated in cellulose sulfate and were angiographically placed into the tumour vasculature of the patients. Cytochrome P450 enzymes produced by these cells then activated a chemotherapeutic agent ifosfamide, and thus facilitated the pancreatic tumor therapy [45].

Regenerated cellulose proved to be permeable for insulin secreted by the islet cells [116]. Implantation of chambers with membranes of regenerated cellulose, containing Langerhans islets, into the retroperitoneal space of rats with experimental diabetes significantly lowered or even normalized the glycemia in these rats [117]. Even cellophane,i.e., a thin sheet made of regenerated cellulose, has been tested for potential islet wrapping [118].

Other materials experimentally used for pancreatic islet encapsulation and long-term cultivation were methylcellulose, applied in porcine islets [119] and carboxymethylcellulose, used for xenotransplantation of rat islets into mice [120, 121] and mouse islets into rats [122]. In these cases, carboxymethylcellulose was used as a component of multilayered capsules, which also contained agarose, polystyrene sulfonic acid and polybrene [120-122]. A cellulose molecular dialysis Spectra/Por 2 membrane was also succesfully tested as a potential candidate for pancreatic islet encapsulation [123].

Cellulose acetate and cellulose acetate phthalate were interesting materials. Cellulose acetate was used for constructing a microsensor for measuring the concentration of oxygen around and within pancreatic islets, which is needed for adequate insulin secretion [124]. Cellulose acetate phthalate proved to be an efficient antimicrobial agent, capable of blocking the infection of human immature Langerhans cells with HIV-1 virus [125].

Cellulose and Nervous System Regeneration

Cellulose-based materials have been widely used for repairing and regenerating peripheral nerves and also for the central nervous system, namely spinal cord and brain. These applications began relatively early,i.e., at the end of the 1980s.

For the peripheral nerves, the pioneering experiments were performed *in vitro* on nitrocellulose in the form of paper soaked with basic fibroblast growth factor, known as mitogen for endothelial cells, fibroblasts and Schwann cells,i.e., cell types present in the peripheral nerve [126], or on nitrocellulose in the form of blots containing neuronotrophic factors [127]. The nitrocellulose paper was applied in experiments *in vivo* for anastomosis and regeneration of transected sciatic nerves in mice [128]. Other constructs applied for experimental sciatic nerve regeneration included methylcellulose gels loaded with platelet-derived growth factor and insulin-like growth factor-I [129, 130], a carboxymethylcellulose vehicle with a pyrimidine analog Xymedon [131], and particularly woven oxidized regenerated cellulose gauze with amniotic fluid mesenchymal stem cells [132]. Also lignin, a macromolecule crosslinking various plant polysaccharides, including cellulose, induced differentiation of embryonic stem cells into neuroectodermal cells, namely ocular cells and neural cells [133].

Electroactive substances or dynamic cultivation enhanced the supportive effects of cellulose-based materials on nerve regeneration. Loading porous cellulose aerogels with electrically conductive polypyrrole nanoparticles enhanced the adhesion, proliferation and neurite extension in rat pheochromocytoma PC12 cells, which have neuroblastic potential [134]. Aligned synthetic microfiber scaffolds of viscose rayon and electrospun polystyrene have been used for establishing an advanced dynamic bioreactor for tissue engineering of peripheral nerve conduits *in vitro*. Schwann cells seeded on these scaffolds and exposed to continuous medium flow increased in number markedly compared to a static culture [135].

Cellulose-based materials also promoted peripheral nerve regeneration by preventing perineural scar formation. In experiments *in vivo* performed on rats and rabbits, composites of hyaluronic acid and carboxymethylcellulose in the form of an injectable solution [136] or membranes [137], and also bacterial cellulose in the form of tubes [138], supported nerve regeneration by preventing infiltration of the perineural tissue with inflammatory cells, by preventing excessive proliferation of connective tissue cells, and also by accumulating neurotrophic factors in the site of injury.

In spine surgery, nitrocellulose was the first cellulose-based material to be used for regenerating injured dorsal roots. Intraspinal implantation of nitrocellulose carrying the nerve growth factor promoted axonal regrowth from the damaged roots in rats [139]. Another material that was applied at an early stage in spine surgery was carboxymethylcellulose, which prevented epidural scar formation following laminectomy in rabbits [140]. Another cellulose-based material used for preventing epidural fibrosis and adhesion was carboxymethylcellulose with polyethylene oxide (Oxiplex/SP Gel, FzioMed, Inc., San Luis Obispo, CA), which was clinically applied in patients who underwent surgery for unilateral herniation of the lumbar discs and suffered from severe leg pain and lower-extremity weakness [141]. A hydrogel material composed of poly(2-hydroxyethyl methacrylate-co-methyl methacrylate (pHEMA-MMA) with guidance channels containing methylcellulose improved axonal regeneration after complete spinal cord transection in rats [142]. A hydrogel blend of hyaluronan and methylcellulose, covalently modified with recombinant rat platelet-derived growth factor-A was applied as an injectable carrier for delivering adult brain-derived neural stem/progenitor cells (NSPCs) into rats with an experimental spinal cord injury. These rats then showed improved behavioral recovery, a significant reduction in cavitation, improved graft survival, increased oligodendrocytic differentiation, and sparing of perilesional oligodendrocytes and neurons [143]. Other injectable hydrogels suitable for delivering cells and neurotrophing into the injured spinal cord are based on poly(N-isopropylacrylamide) (PNIPAAm), lightly cross-linked with polyethylene glycol or methylcellulose [144].

Attempts to regenerate damaged brain tissue also started with nitrocellulose. Nitrocellulose soaked with a conditioned medium originating from regenerating fish optic nerves was implanted into rabbits in order to regenerate an experimental injury of the optical nerve, which is anatomically considered as an extension of the brain tissue [145]. Other promising materials include methylcellulose-based materials. Injectable methylcellulose hydrogels proved to be suitable substrates for the repair of an experimental brain injury in rats [146]. Hyaluronan-methylcellulose composite hydrogels were used as carriers for local delivery of erythropoietin, epidermal growth factor and Cyclosporin to mouse brain in order to repair the tissue damage after a stroke by means of activating endogenous neural stem and progenitor cells, maintaining their viability and reducing the inflammatory response to the stroke [147-149]. Oxidized cellulose also holds potential for treating brain damage. Oxidized regenerated cellulose in fibrillar form significantly reduced bleeding in hemorrhagic cerebral contusions in rats, and its effect was comparable to that of fibrillar collagen [150].

Cellulose-based materials were also effective in repairing damaged *dura mater* on the brain. Biosynthetic cellulose,i.e., a Brazilian-manufactured membrane used in plastic surgery as a temporary skin substitute for second degree burns, has also been applied for treating experimental defects of the *dura mater* covering the brain or spine in dogs and sheep [151, 152]. Biosynthetic cellulose grafts have been applied clinically in human patients as dural

replacements [46]. Other clinically-applied cellulose materials include oxidized cellulose reinforced by fibrin glue, which has been used for sutureless closure of minor dural defects [47]. Bacterial cellulose is another material that is promising as a dural substitute [153].

Cellulose and Urinary System Reconstruction

Similarly as in neural tissue regeneration, the use of cellulose in surgery, repair and regeneration of the urinary system started as early as the 1960s and 1970s, when oxidized cellulose was used as hemostatic agent after experimental nephrectomy [154, 155]. Oxidized cellulose was also tested for prevention of a urinary fistula after laparoscopic partial nephrectomy [156], and for closing a bladder neck fistula complicated by urethral and vaginal stenosis [157]. Microporous scaffolds made of bacterial cellulose and seeded with human urine-derived stem cells supported the formation of a multilayered urothelium, which expressed urothelial differentiation markers uroplakin Ia and cytokeratins AE1/AE3. These constructs thus hold promise for forming tissue-engineered urinary conduits for urinary reconstruction and diversion [158].

Cellulose acetate in the form of porous membranes has been applied for constructing a bioartificial renal tubule system. This system promoted the proliferation and functional differentiation of Lewis-lung cancer porcine kidney 1 (LLC-PK1) cells, manifested by the expression of glucose transporters, which was not observed on conventional nonporous polystyrene plates [159]. Cellulose acetate in the form of electrospun porous microfibrous three-dimensional scaffolds has been tested for potential reconstruction of the urinary bladder [160].

Cellulose and Bone Tissue Engineering

Cellulose shows great potential in bone tissue engineering, mainly due to its biocompatibility and its ability to promote osteoblast proliferation and osteogenic cell differentiation [161, 162]. However, it has usually been tested as a part of more complex composites, since cellulose itself does not have the necessary mechanical strength for load-bearing applications [163-166]. One of the most widely-used materials in bone tissue engineering is hydroxyapatite (HAp), which has been applied in combination with cellulose to improve its mechanical properties [164-168].

Liuyun et al. [164] developed composite membranes of chitosan-carboxymethylcellulose polyelectrolyte filled with nano-HAp. The addition of nano-HAp to the cellulose-based membranes improved their microstructure compatibility, mechanical properties, swelling behavior, their degradation and bioactivity *in vitro*, when compared to membranes without HAp. The most appropriate mechanical properties were achieved when 40 wt.% of nano-HAp was used. Accordingly, the research group of Jiang et al. [166] investigated membranes composed of chitosan, sodium carboxymethylcellulose and nano-HAp. Osteoblasts cultivated on the membrane with 60 wt.% of nano-HAp exhibited the highest cell viability and osteocalcin expression. Moreover, an important role in this study was played by the spiral-cylindrical arrangement of the scaffold, which promoted complete infiltration with bone tissue *in vivo*. Another study investigated bacterial cellulose (BC) supplemented with HAp,

and concluded that there was significantly increased osteoblast adhesion and growth on BC/HAp membranes as compared to BC alone, and also greater bone nodule formation and mineralization [167].

Cellulose-based hydrogels form another group that has been widely tested in bone tissue engineering. The use of hydrogels for bone regeneration is problematic due to their low modulus to support cell adhesion and proliferation. Carboxymethylcellulose-HAp hybrid hydrogel was evaluated by using human osteoblast-like MG-63 cell line. Addition of HAp to the hydrogel enhanced the cell proliferation and promoted the production of mineralized extracellular matrix [168]. Another study developed multifunctional polysaccharide hydrogels composed of methylcellulose, chitosan and agarose. Their stiffness was increased by crosslinking the chitosan with increasing amounts of genipin. A positive correlation was found between increasing gel stiffness and increasing osteoblast and fibroblast proliferation [169]. In addition, studies suggested that cellulose-based hydrogels promote differentiation of human mesenchymal stem cells into osteoblastic phenotype [170], or promote mineralization of regenerated cellulose hydrogel surfaces by human bone marrow stromal cells [171]. Successful osteogenic differentiation of human adipose-derived stem cells was also achieved on bacterial cellulose [162].

Another way to approach mineralization of the cellulose surface is indirectly *via* immersion of cellulose in simulated body fluid (SBF). Experiments resulted in effectual coverage of cellulose with hydroxyapatite crystals after 14 days of immersion. Subsequently, the adhesion and growth of osteoblast cells was improved on mineralized cellulose scaffolds [172]. Another recent study used electrospun hydroxyethyl cellulose/polyvinyl alcohol nanofibers and immersed them in concentrated simulated body fluid, resulting in nanofibers coated in bone-like apatite [173].

Our studies have also contributed to knowledge on the mineralization of cellulose scaffolds in SBF and their potential further use for bone tissue engineering. Plant-derived cellulose in the form of cotton fabrics, viscose fabrics, and also 6-carboxycellulose with 2.1 wt.% of -COOH groups proved to be suitable for vascular tissue engineering [49, 50]. These samples were mineralized in SBF with or without citric acid, which acts a modulator of the mineralization of various materials in SBF. For example, in the concentration range from 0.3 to 2 mM in SBF, citric acid promoted the mineralization of collagen membranes [174]. In our experiments, all three cellulose-based materials promoted cell adhesion and subsequent growth, though the cells adhered in relatively low initial densities on day 1 after seeding (Figure 1 A-C). Nevertheless, the cells were able to spread and to gain a spindle-shaped morphology, oriented along the fibers (Figure 1 D-F) and to proliferate within a period of 6 days. The final cell number on day 6 was highest on viscose and lowest on 6-carboxycellulose with 2.1 wt.% of -COOH groups. The cell number was usually higher on the materials mineralized in SBF with citric acid than on the non-mineralized materials and on the material mineralized without citric acid, which was most apparent on 6-carboxycellulose with 2.1 wt.% of -COOH groups (Figure 1 A-C). This could be explained by higher mineralization of the materials due to hydrogen bonding of citric acid to the cellulose cloth and its ability to form chelates with calcium ions [175].

The properties of cellulose materials can also be improved and made suitable for bone tissue engineering applications by functionalizing them with various bioactive agents, e.g., functionalization of bacterial cellulose with osteogenic growth peptide [176], or by modifying

the cellulose itself chemically to achieve more desired properties, for example by sulfation, carboxylation or carboxymethylation [177]. The results of Peschel et al. [177] showed that sulfated or carboxylated cellulose had some osteogenic activity, while carboxymethylated cellulose has none. A similar study compared cellulose and sulfated cellulose fibrous meshes, and concluded that the sulfated fibrous mesh more readily supported the attachment and osteogenic differentiation of rat bone marrow stromal cells. It also showed better retention capacity for bone morphogenetic protein-2 (BMP-2) than the pure cellulose mesh [178].

A reversed approach uses cellulose as a functionalizing or coating agent to improve the properties of various other materials, e.g., functionalization of titanium oxide surfaces with phosphated carboxymethylcellulose [179] or coating polyethylene terephthalate with hydroxypropylcellulose [180]. Both these coatings improved osseointegration with the host tissues.

Cellulose was also tested as a potential drug-delivery system to improve bone defect sites, for example by loading bacterial cellulose with BMP-2. BC loaded with BMP-2 induced differentiation of seeded mouse fibroblast-like C2C12 cells into osteoblasts, and the osteogenic activity was positively correlated to the concentrations of BMP-2. This study was also extended with an *in vivo* experiment, which showed more bone formation and higher calcium concentration in subcutaneous implantation sites when BC with BMP-2 was implanted than when BC alone was used [181]. Another research group developed ethyl cellulose microspheres that were loaded with model drug Ceftazidime. These microspheres were then incorporated in an HAp/polyurethane composite scaffold. This material proved to be an effective cytocompatible drug delivery system with antibacterial properties and with retained release of the drug for up to 60 days [182].

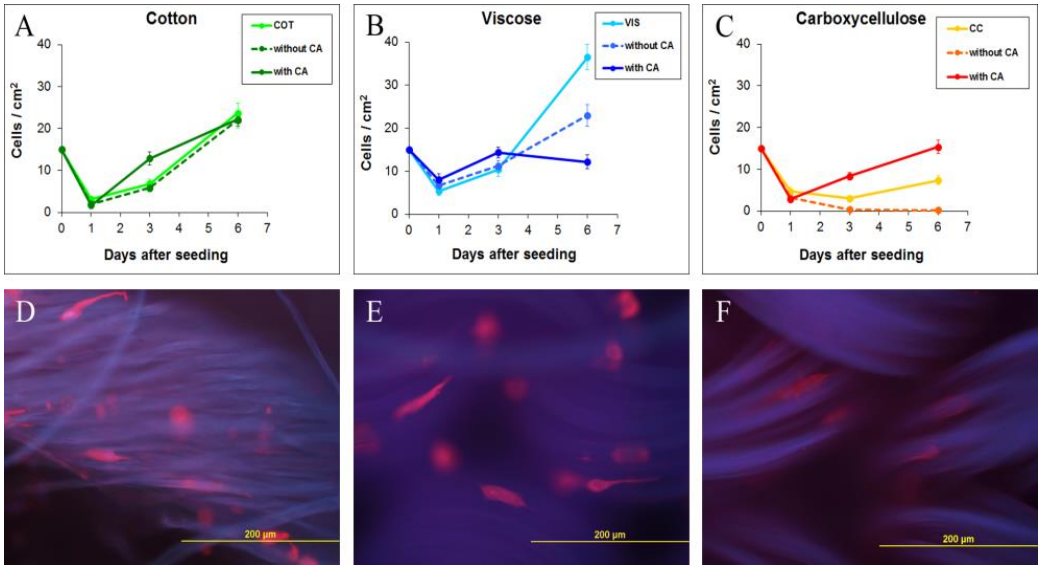

Figure 1. Growth curves (A-C) and morphology (D-F) of human osteoblast-like MG-63 cells in cultures on cotton (A, D), viscose (B, E) and 6-carboxycellulose with 2.1 wt.% of – COOH groups (C, F). A-C: cotton, viscose and 6-carboxycellulose (COT, VIS and CC, repectively) were mineralized with or without citric acid (CA). D-F: Cells on day 1 after seeding; stained with Texas Red C_2-maleimide (red; stains proteins of the cell membrane and cytoplasm) and Hoechst #33342 (blue; stains the cell nuclei and also tints the materials). Olympus IX 71 microscope, obj. 20, digital DP 70 camera, bar = 200 µm.

Scientists agree that a certain degree of microporosity within the scaffold is beneficial to osteoblast ingrowth and subsequent mineralization of the scaffold [183, 184]. Zaborowska et al. [183] compared nanoporous and microporous bacterial cellulose scaffolds, which were seeded with MC3T3-E1 osteoprogenitor cells. The cells formed denser mineral deposits on microporous scaffolds than on nanoporous scaffolds. The micropores in this study ranged between 300-500 μm. Another research group developed nanofibrous cellulose scaffolds with laser induced micropores ranging between 50-300 μm that enhanced osteoblast attachment at the edge of these pores [184]. However, bigger pores ranging up to 750 μm formed in cellulose matrix were also shown to be effectual for osteoblast adhesion and growth [172].

Although most studies with cellulose scaffolds are held as *in vitro* tests, there have also been several *in vivo* experiments. Besides the studies already mentioned above, which were performed partially *in vivo* [164, 181], there was a study which tested the biological properties of bacterial cellulose-HAp membranes. These membranes were implanted in non-critical bone defects in rat tibiae. After 4 weeks, the defects were filled with new bone [165]. Using a subcutaneous implantation rat model, an analysis was made of a new injectable material composed of beta-tricalcium phosphate, methylcellulose and hyaluronic acid. Tissue reaction with the implant resulted in increased vascularization and a longer *in vivo* lifetime in comparison with implants consisting of beta-tricalcium phosphate alone [185]. A long-term experiment lasting for 36 months evaluated calcium phosphate bone cement with carboxymethylcellulose implanted in vertebral bone defects of the sheep. On average, after 36 months the defect section consisted of approximately 14% bone, 82% cement, and 4% bone marrow, with no fibrous tissue [186]. A later study used the sheep model in a similar manner [187]. This study evaluated the reaction of tissue to oxidized cellulose scaffolds and compared it with the reaction of tissue to collagen scaffolds. No significant difference was found between the two materials in the rate of repair of the bone defects, which were completely repaired by lamellar bone at 6-8 weeks.

An injectable bone substitute was also applied clinically for filling bone defects in patients after tooth extraction. This substitute was prepared by suspending biphasic calcium phosphate microparticles in a water-soluble cellulose polymer carrier phase. Three years after surgery, small biopsies of the implanted areas revealed gradual substitution of the filler by bone tissue and preservation of the height of the alveolar bone crest [48].

Cellulose and Cartilage Tissue Engineering

Cellulose has been used for engineering various types of cartilage, e.g., articular cartilage, meniscus, intervertebral discs and auricular cartilage. Cartilage tissue engineering is considered difficult due to the fact that cartilage is an avascular tissue with very low spontaneous regeneration potential. In addition, chondrocytes are prone to dedifferentiation towards fibroblast-like cell phenotype. Last but not least, the cartilage replacement should meet relatively high requirements for mechanical resistance, particularly in load-bearing applications.

For engineering articular cartilage, cellulose and its derivatives and composites have often been used in the form of injectable hydrogels with encapsulated cells in order to fill the cartilage tissue defects with minimal invasion and pain. The cellulose-based hydrogels included, e.g., silanized hydroxypropyl methylcellulose [188, 189], and particularly

thermoresponsive polymers, such as chitosan-beta glycerophosphate-hydroxyethyl cellulose [190, 191] or poly(N-isopropylacrylamide)-g-methylcellulose [192]. These polymers are characterized by a sol-gel transition at 37°C, which enables them to be injected in their liquid state and then to gel at body temperature. Amidic derivatives of carboxymethylcellulose have also been developed in order to mimic the advantageous physicochemical properties of hyaluronan while overcoming its excessively fast degradation time [193]. For better mechanical strength, hydrogels have been reinforced with multi-wall carbon nanotubes [194] or by induction of a fibrous component, e.g., by critical point drying in a bacterial cellulose hydrogel [195]. In some hydrogels, pores were created in order to facilitate the penetration of cells inside the scaffolds, e.g., bacterial nanocellulose hydrogels perforated using a laser [196].

Cellulose-based materials for articular cartilage engineering have also been constructed, primarily in the form of fibrous, porous or combined scaffolds, such as nanofibrous bacterial cellulose [197], composites of nanofibrous bacterial cellulose and poly(vinyl alcohol) matrix [198], bacterial cellulose scaffolds with pores created by extrusion of wax particles [199], cellulose and cellulose/recombinant type II collagen sponges [200], and composites containing cellulose nanofibers, derived from rice straw and coated with a starch film, in which the pores were created by a salt leaching technique [201]. Cellulose-based scaffolds were also used for engineering the osteochondral interface, e.g., asymmetric porous composites consisting of cellulose acetate matrix and bioactive glass particles, prepared by phase separation techniques [202], or non-woven cellulose fabrics activated in a saturated $Ca(OH)_2$ solution and subsequently coated with a calcium phosphate layer precipitated from a supersaturated physiological solution [203].

Injectable cellulose-based hydrogels, particularly photocrosslinked carboxy-methylcellulose hydrogels with encapsulated cells, are also applicable for engineering nucleus pulposus, an important component of intervertebral discs [204-206]. Bacterial cellulose has been used for replacing auricular cartilage and meniscus. Bacterial cellulose is composed of highly hydrated nanofibrils (99% water) with high mechanical strength. Bacterial cellulose with an increased cellulose content of 17% exhibited mechanical strength similar to that of auricular cartilage, a minimal cytotoxic response *in vitro*, tested on murine fibrosarcoma L929 cells, and a minimal foreign body response *in vivo*, evaluated after intradermal implantation into rabbits [207]. Bacterial cellulose can also be fabricated into patient-specific auricular shapes [208]. In the case of meniscus replacement, the Young's moduli of bacterial cellulose gel and pig meniscus were similar in magnitude under a compression load of 2 kPa and had five times better mechanical properties than a reference collagen material [209].

For encapsulation into hydrogels or for seeding on various scaffolds, chondrogenic cells were used in the form of cell lines, such as murine ATDC5 cells [192] or differentiated chondrocytes derived from articular cartilage [190, 193]. This approach is appropriate for testing the materials in vitro, but for *in vivo* testing or for potential clinical application, a more advantageous approach is to use autologous nasal chondrocytes [188] or autologous mesenchymal stem cells derived from bone marrow [191, 195, 206] or from adipose tissue [189]. Cells derived from nucleus pulposus are often applied for engineering intervertebral discs [204, 205]. Chondrogenic cell differentiation is supported by transforming growth factor β_3 added into the cell culture medium or released by the scaffolds [191, 206]. Some specific scaffold components, such as addition of silk to cellulose, have also been reported to support

chondrogenic cell differentiation [210]. This differentiation is manifested by the expression of COL2A1, ACAN, SOX9, and COMP genes and by synthesis of cartilage-specific components of extracellular matrix, namely aggrecan and type II collagen [188, 189, 206, 211]. In order to make cellulose-based scaffolds degradable, novel bacterial cellulose was formed in metabolically engineered *Gluconacetobacter xylinus*. This cellulose is lysozyme-susceptible, and can gradually be replaced by newly formed regenerated cartilage tissue [211].

Cellulose for Tendon and Ligament Repair

The first application of cellulose-based materials in tendon surgery was to prevent the formation of adhesions between the healing tendon and the surrounding structures, such as bone, muscle, skin, tendon sheath, or other tendons, via scar tissue. For these purposes, the following formulation was tested in rabbits and rats: Interceed TC7 (Johnson, Johnson, USA), which is a fabric comprised of oxidized regenerated cellulose [212, 213], and Seprafilm Bioresorbable Membrane (Genzyme Corporation, Cambridge, MA), containing sodium hyaluronate and carboxymethylcellulose [214]. It was concluded that these materials significantly reduced peritendinous adhesions in experimental animals and are promising for clinical application in human patients.

Cellulose-based materials have also been used for osseointegration of ligament replacements and for ligament and tendon tissue engineering. For example, hydroxypropyl cellulose coating of polyethylene terephthalate artificial ligaments enhances graft osseointegration in the bone tunnel in the proximal tibia of rabbits [215]. Fibrous networks of cellulose nanofibers and collagen crosslinked using a bio-based crosslinker genipin promoted the adhesion, growth and differentiation of human ligament cells and human endothelial cells. In addition, the material showed mechanical performance similar to that of the natural ligament and tendon [216]. Similar results were obtained with electrospun cellulose nanofibers, which improved their tensile strength, elastic modulus and thermal stability after being reinforced with cellulose nanocrystals, and supported the adhesion and oriented growth of human fibroblasts [217].

Cellulose-based materials are also promising for periodontal ligament repair. Suspensions of nano-hydroxyapatite/sodium carboxymethylcellulose in cell culture medium accelerated the proliferation of human periodontal ligament cells (HPLCs) by shortening the G1 phase of the cell cycle [218]. When HPLCs were cultured on a polyelectrolyte complex consisting of chitosan and sulfated cellulose, treatment with 10^{-7}-10^{-9} M dexamethason promoted HPLC growth and inhibited the production of cell aggregates [219].

CONCLUSION AND FURTHER PERSPECTIVES

Cellulose is the most abundant biopolymer on Earth, and it accordingly also has abundant biomedical applications, including clinical applications. Cellulose has been used for repairing, regenerating and reconstructing practically all of the tissues in the mammalian organism. In its clinical applications, however, cellulose supports the healing and the function of tissues

and organs rather indirectly, e.g., by covering wounds and releasing drugs into them, by preventing postoperative adhesions, by hemostasis, hemodialysis or by covering and filling various tissue defects. Direct clinical applications of cellulose-based materials as scaffolds for tissue engineering and cell delivery are still relatively rare, although extensive research has been carried out in this field under *in vitro* and *in vivo* experimental conditions. The practical use of cellulose-based materials in tissue engineering and in cell therapies now needs to be promoted. For this purpose, cellulose-based scaffolds can be tailored by a wide range of modifications, including combinations of cellulose with other natural or synthetic polymers, ceramics, metals or carbon materials, loading with bioactive molecules (drugs, growth factors), functionalization with various chemical groups, oxidation, preparation of scaffolds with various morphologies, etc. All these modifications can control the physical and chemical properties of cellulose-based scaffolds, which are essential for cell-material interaction. They can also enhance the degradability of the material, which is an important property for advanced tissue engineering, in which material scaffolds act as a temporary support for the formation of new tissue.

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